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# Brief Synopsis of EPA Office of Research and Development and the Health Effects Institute Mobile Source Work

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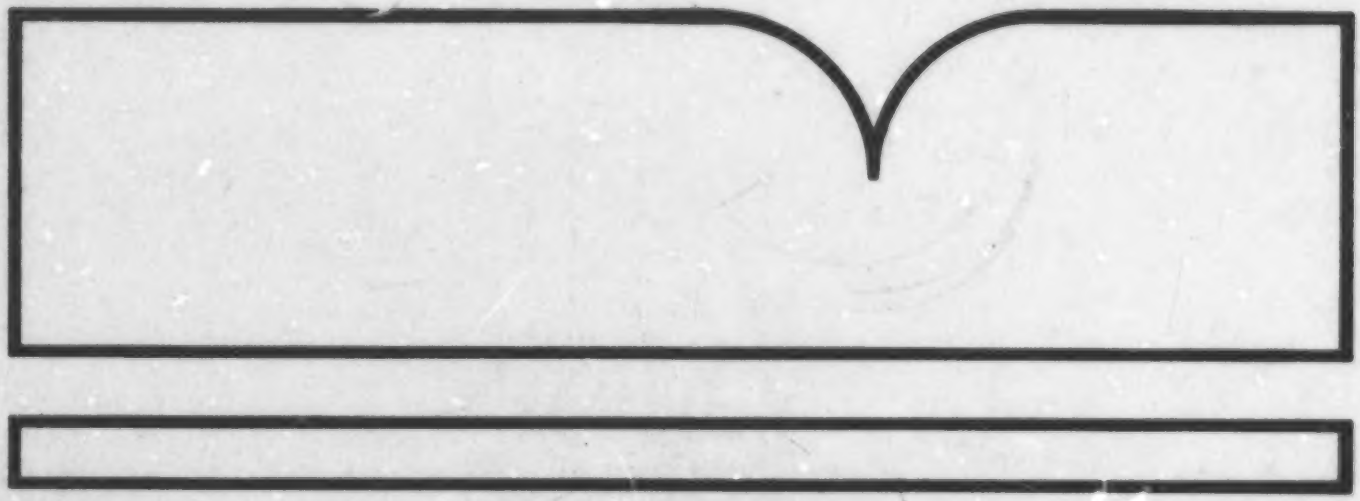

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16. ABSTRACT

The purpose of this report is to cover the Office of Research & Development (ORD) mobile source work. This report will summarize the work in progress by ORD and give some of the pertinent results. This report will also briefly metion future work planned by ORD.

Some of the important areas metioned are; chemical characterization work, development of method to collect gas-phase hydrocarbons in diesel exhaust, effect of NO2 in mutagen artifact generation, study of filter efficiency and identification of types of compounds responsible for Ames test activity in diesel particulates.

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May, 1981

Control Technology Assessment and Characterization Branch
Emission Control Technology Division
Office of Mobile Source Air Pollution Control
Office of Air, Noise and Radiation
U.S. Environmental Protection Agency
2565 Plymouth Road
Ann Arbor, Michigan 48105

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Summary

The EPA Office of Research and Development has many important mobile source projects in progress as discussed in this paper while the Health Effects Institute will begin work shortly. Some of the more important items that can be noted at this point are given below.

1. ORD-ESRL has developed a preliminary method to collect gas phase hydrocarbon samples from gasoline and Diesel vehicles for bioassay testing. This method involves use of XAD-2 cartridge traps and is presently being written up. After the method is available later this year, work can start to make an assessment of the mutagenicity of the gas phase samples relative to the particulates.

2. ORD-ESRL has done a number of tests to determine if $NO_2$ in the exhaust gas can cause artifact mutagen formation on the particulate filter and thus an artificially high Ames test response. Preliminary results indicate that no artifact will be formed provided that the $NO_2$ levels in the diluted exhaust are below 5 ppm which is usually the case.

3. Significant work is being done to identify the compounds responsible for the mutagenic activity in Diesel exhaust. Numerous oxygenated polynuclear aromatic hydrocarbons and nitroaromatics have been identified as being responsible for much of the mutagenic activity.

4. Additional light duty Diesel and gasoline vehicles have been tested. Particulate emissions and Ames test response have been measured. Also, several heavy duty Diesel engines have been tested. Much work will be done on various synthetic fuels derived from coal and oil-shale in the future.

5. ORD-EMSL has some short term monitoring work underway to measure personal exposure to CO and correlate it to the readings taken at fixed site NAAQS monitors. This work involves contractors measuring CO levels along commuting routes and other places (e.g. central business district and outlying areas).

6. A longer term CO monitoring project involving a large number of people wearing CO monitors should be started soon. Their CO exposure will be correlated to their personal activity and to readings obtained at the fixed site NAAQS monitors.

7. Short term bioassay results have been obtained on a number of Diesel and other samples including coke oven, cigarette smoke condensate, and roofing tar emissions.

8. Skin tumorigenesis initiation data are available on Diesel particulate samples and indicate these samples are less potent than coke oven samples. Intraperitoneal injection results should be available shortly but the intratracheal instillation results will not be available until 1983.

9. Results so far on whole animal inhalation work with Diesels have been generally negative with the exception of some sister chromatid exchange work with hamsters. The sister chromatid exchange work showed a linear relationship between Diesel exposure and the sister chromatid exchange response.

10. ORD-HERL has started a clinical study to establish conclusively the relationship between CO exposure and the decrease in time until onset of chest pain for people with angina pectoris (a heart condition) during exercise. Also, animal work is starting to determine the effect of CO exposure on fetuses. Results for the clinical and animal studies will be available in 1982.

11. ORD-HERL is doing work to develop a tier bioassay test to be used for mutagenic and carcinogenic substances for OMSAPC regulatory efforts under Section 211 (fuel and fuel additives) and Section 202 (a)(4) (assuring motor vehicle systems being certified do not cause or contribute to an unreasonable risk to public health or welfare) of the Clean Air Act. The tier test protocols are expected to be available in late 1982.

12. A revised risk assessment estimating the potential carcinogenic impact of Diesel exhaust emissions is being prepared by ORD and should be available shortly. Meanwhile, ORD has concluded that the London Transit Worker study (a Diesel epidemiology study) is not adequately sensitive to show there would be no excess cancer deaths resulting from the projected exposure to Diesel exhaust.

13. The newly organized Health Effects Institute is an independent organization designed to conduct health research on motor vehicle pollutants. The Institute is currently planning the programs they plan to conduct; EPA and the motor vehicle industry will be submitting a list of suggested program areas to the Institute.

## I. Introduction

For a number of years, the EPA Office of Research and Development (ORD) has conducted a number of mobile source research programs. The initial programs were characterization ones done by the Environmental Sciences Research Laboratory (ESRL) which were followed in about 1975 by mobile source programs in both the Health Effects Research Laboratory (HERL) and the Environmental Monitoring and Support Laboratory (EMSL).

In 1978, EPA started the Mobile Source Research Committee (MSRC) to help assure that ORD mobile source work is responsive to the needs of the Office of Mobile Source Air Pollution Control (OMSAPC). Since that time, a number of problems have surfaced. OMSAPC feels that ORD will sometimes implement work ORD feels is needed rather than work specifically requested by the program office. ORD will frequently do some projects that OMSAPC disapproves since OMSAPC feels these projects do not meet OMSAPC needs. Also, there have been problems in transmitting results of ORD research to OMSAPC. On the other hand, ORD frequently feels that some work requested by OMSAPC is either not scientifically valid or too short term and of a service-type function (e.g. run 300 samples for Ames test activity) rather than longer term research. Even with these problems, there is considerable ORD mobile source work that is useful to OMSAPC. Also, ORD and OMSAPC are trying to resolve the problems experienced to date.

The purpose of this report is not to discuss any problem areas between OMSAPC and ORD but instead to cover the ORD mobile source work itself. This report will summarize the work in progress by ORD and give some of the pertinent results. This report will also briefly mention future work planned by ORD.

The results and work discussed in this paper are that available to OMSAPC as of April 10, 1981.

## II. Chemical Characterization Work

The chemical characterization work for unregulated mobile source emissions is done by ESRL. This program involves developing methods to measure unregulated emissions and conducting extensive Diesel characterization work.

### A. Development of Method to Collect Gas-phase Hydrocarbons in Diesel Exhaust

One of the highest priorities expressed by OMSAPC was to develop a method to collect artifact-free samples of gas phase hydrocarbons in motor vehicle exhaust for bioassay testing. ESRL has evaluated both a filter cartridge and a condensate method.

Preliminary tests have been done with a method involving filtering the exhaust particulates and then condensing components in the gas stream. The

condensate appears to have low Ames test activity. If the filter upstream of the condenser is removed, the condensate will contain some Diesel particulate and has somewhat higher Ames test activity.

The filter cartridge method involves passing a gas stream sample after a conventional particulate filter through a cartridge or bed of treated XAD-2 resin. After the gas stream is passed through the XAD-2, the hydrocarbons absorbed onto the XAD-2 are removed from the resin by methylene chloride extraction. The lower molecular weight hydrocarbons (e.g. below C-10) are sufficiently volatile that they are probably lost during the extraction. However, hydrocarbons above C-10 are retained and can then be subject to the Ames test. Since the conventional particulate filter will generally retain hydrocarbon compounds above C-15, the XAD-2 traps could provide a good method to collect hydrocarbons in the C-10 to C-15 range.

A very preliminary result of Ames testing on the gas phase hydrocarbon collected by this method for a VW Diesel Rabbit shows that the activity may be low compared to that of the particulate.

The methods and results mentioned above are very preliminary and have not yet been published by ORD. However, OMSAPC has requested that ORD publish these results with a full description of the method as soon as possible.

In the past, ORD has expended great effort in identifying the hydrocarbon compounds in the gas phase of Diesel and gasoline vehicle exhaust. This work has been described by ORD in several publications and was recently summarized in an OMSAPC report.[1]

B. Potential Effect of $NO_2$ in Mutagen Artifact Generation

EPA has been concerned that high levels of $NO_2$ in the dilution tunnel may result in a reaction of $NO_2$ with some of the hydrocarbons present either in the gas stream or on the particulate filter. The reaction products including various nitroaromatic compounds could result in an increased Ames test response. Since these reaction products from the hydrocarbons and $NO_2$ would not be expected to form in the atmosphere where these compounds would be greatly diluted, the Ames test response would be artifically high.

ORD has run some experiments where additional $NO_2$ has been introduced into the dilution tunnel. These experiments show that artifact is created in Diesel particulates if the $NO_2$ levels are above 5 ppm. ORD plans to publish these results shortly.

A concern has also been raised that lower levels of $NO_2$ may result in artifact formation although this artifact formation would probably be lower than that which might be experienced at higher $NO_2$ levels. OMSAPC has run some experiments with a single cylinder Diesel engine with artificial combustion air containing no nitrogen and a nitrogen free fuel. In this experiment, no NOx would be formed in the exhaust gas. By comparing the results of these tests to tests with conventional air (80% $N_2$, 20% $O_2$) and regular Diesel fuel (which contains traces of nitrogen), one can determine if there were artifact formation due to the $NO_2$. The results of these tests have not yet been published. Also, investigators at Penn State University and John Hopkins University plan to repeat these tests in the coming year.

C. Study of Filter Efficiency

ORD has just completed a study[2] examining various filters that can be used for Diesel particulate collection. The filters investigated were Millipore, Fluoropore FA, Dexiglas, Gelman A-E, Ghia Zefluor lu, Pallflex T60A20, Pallflex TX40HI, and Pallflex 400HO.

A common back-up filter was used to measure any break-through from the test filter. It was found that all of the filters collected greater than 95% of the particulate on the primary filter as determined by the standard EPA method[3]. However, it was noted that small differences in collection efficiencies occurred in the cold start bag of the Federal Test Procedure rather than the portions when the vehicle engines were warmed-up.

Even though all of these fiters may be acceptable for determining total particulate mass, it is not known if an interaction of the filter and the particulate such as can occur with glass fiber filters would affect the Ames test results.

D. Identification of Types of Compounds Responsible for Ames Test Activity in Diesel Particulates.

ORD has spent effort in separating the methylene chloride extract of Diesel particulates by high pressure liquid chromatography. Three major fractions have been obtained: non-polar, moderately polar (transition), and highly polar. Some of this work has been done in a cooperative scientific project with Ford Motor Company.

The non-polar peak consists of aliphatic and polynuclear aromatic hydrocarbons (many of them alkyl substituted) which are not responsible for a major portion of the Ames test activity.

The transition and polar fractions account for most of the Ames test activity. About 50% by weight of the material in the transition fraction consists of various oxygenated polynuclear aromatic hydrocarbons (including hydroxy, ketone, carboxaldehyde, quinone, acid anhydride, and nitro compounds). Three specific nitro compounds including 1-nitropyrene have been identified. A total of about 80 other compounds have also been identified. The remainder of the transition fraction is thought to be contaminants introduced in the analysis procedure. The polar fraction consists of carboxylic acid polynuclear aromatic hydrocarbons which also have significant Ames test activity.[4]

It appears that a great number of compounds may be responsible for the Ames test activity.

E. Particulate Emission Factors From Light and Heavy Duty Vehicles

OMSAPC expressed a need for additional particulate emission data from both gasoline and Diesel vehicles.

Tests were run in-house at ESRL on a 1980 VW Diesel Rabbit and Oldsmobile Diesel. Some tests were done with the VW under malfunction conditions. The table below summarizes some of the most pertinent results.

Table 1
ESRL Tests on VW and Oldsmobile Diesel

| Vehicle | HC g/mile | CO g/mile | NOx g/mile | Particulate g/mile | Soluble Organics % |
|---|---|---|---|---|---|
| VW normal | 0.51 | 1.31 | 0.99 | 0.43 | 27 |
| VW - a | 0.68 | 1.69 | 1.23 | 0.54 | |
| VW - b | 0.74 | 1.70 | 1.28 | 0.76 | |
| VW - c | 0.51 | 1.31 | 0.99 | 0.43 | |
| Oldsmobile | 0.25 | 1.24 | 1.54 | 0.54 | 15 |

a timing retarded/bad injector
b bad injector
c new injector

These test results show that a bad injector increases the emissions; similarly specific Ames test activity increased by over 50% on TA98 due to the faulty injector. Tests of both vehicles over differenmt driving cycles showed that Ames test specific activity was affected slightly by the driving cycle: the cycles examined were the FTP, HFET, New York City cycle, and Congested Freeway Driving Schedule[5].

Additional studies[6] were done for EPA by the New York State Department of Environmental Conservation on 19 in-use Diesels at different mileages. These tests showed that various driving cycles, from the limited number of tests run, do not affect Ames test specific activity other than emissions at idle are somewhat less active. These tests show in-use particulate emissions appear to be in the same range as obtained from tests of prototype vehicles. However, the results to date may be too limited to make this a firm conclusion in part since little data at high mileage are available.

ESRL has also conducted a contract study at Southwest Research Institute[7] to measure unregulated emissions from four heavy duty engines (Caterpillar 3208, Mack ENDT 676, Cummins 290, and Detroit Diesel 8V-71) over the newly developed EPA heavy-duty-engine transient cycle. A summary of some of the emissions results is given below.

Table 2
Emissions Results From Heavy-Duty Engines*

| Engine | HC g/mile | CO g/mile | NOx g/mile | Particulate g/mile |
|---|---|---|---|---|
| 1979 Caterpillar 3208 | 1.98 | 5.6 | 18.6 | 0.92 |
| 1979 Mack ENDT 676 | 1.61 | 9.6 | 29.1 | 1.95 |
| 1979 Cummins 290 | 2.37 | 11.2 | 25.4 | 1.64 |
| 1977 DD 8V-71 | 2.25 | 64.9 | 39.1 | 2.74 |

*results obtained over 1983 transient cycle

It was found that the Los Angeles Freeway segment of the transient cycle contibuted most (40-50%) of the particulates collected. It was found that sulfur compounds constituted about 10% of the particulate mass presumably due to sulfate. Also, HCN and $N_2O$ emissions were measured and found to be below 80 mg/mile. Emissions were measured with different fuels; it was found that HC was higher with the minimum quality DF-2 with the 4-stroke engines and benzo(a)pyrene (BaP) emissions also increased with this fuel.

F. Particulate Emissions from In-Use Gasoline Vehicles

ESRL started an in-house program to measure particulate emissions from 20 in-use gasoline vehicles. Of these cars, 16 are catalyst-equipped and thus burn unleaded fuel. Ames tests will be run on the samples collected. Tests have so far been run on 13 of the 20 cars but no results are available yet. A final report on this is due later this year.

G. Synthetic Fuels Work

ESRL has obtained some shale-oil Diesel fuel samples and plans to test some vehicles in-house with them. Both gaseous emissions including gas-phase hydrocarbons and particulates will be analyzed. Some limited work was done with a relatively high quality oil shale derived Diesel fuel showing the Ames test reactivity of the particulates is roughly equivalent to that obtained with conventional fuel.[8] Future ESRL plans call for obtaining H-coal, SRC-II and donor solvent fuels. This work complements work OMSAPC has underway on coal and oil-shale fuels. Also, the ORD Industrial Engineering Research Laboratory (IERL) plans to start a program here.

III. Carbon Monoxide and Other Monitoring Work

EMSL has a number of monitoring projects underway for CO and also does some fuels and quality assurance work. Some of these are short term projects while others are longer term.

A. Carbon Monoxide Work

In the past several years, several studies [9,10,11,12,13,14,15] have been done indicating that CO levels that people are actually exposed to are greater than predicted on the basis of the CO NAAQS fixed-site monitors. In particular, work[14] has shown that the fixed-site monitors underestimate CO exposure for 1 hour periods for Boston commuters by about a factor of 2. OMSAPC has placed very high priority on ORD work to define what actual exposure is to CO. While it is recognized that the EPA NAAQS network for CO is good for determining CO trends and levels at fixed sites, the fixed site network is not as accurate as needed to determine actual exposure.

Another pollutant EPA needs actual exposure data on is Diesel particulates so that more accurate carcinogenic risk assessments can be made. While it is not currently possible to determine actual exposure to Diesel particulates by having people carry Diesel particulate monitors, CO can possibly be used as a surrogate for Diesel particulate exposure. Thus, the CO personal exposure work can possibly be used to refine the Diesel cancer risk assessment.

OMSAPC has requested that ORD implement CO exposure projects in two time frames; a short term project can provide OMSAPC with preliminary data for OMSAPC's more immediate needs while a longer term project can look at the problem in more detail and meet longer term needs.

The short term CO monitoring project is being run in three cities, Stamford, Connecticut, Denver, and Phoenix. ORD contractors are carrying a CO personal monitor while driving over typical commuting routes determined by local transportation data. The monitors are carried along these routes during morning and evening rush hours. An average CO exposure value (e.g. 10 ppm 1 hour average) is determined for certain time periods. Then, during the rest of the day, CO values are obtained by walking around the central business district and driving in outlying suburban areas. Readings are taken in various public buildings and stores.

These data are representative of what CO levels people are exposed to and will be compared with values recorded at the NAAQS monitors. The data should be collected by May 1981 and will be analyzed shortly thereafter. Some results should be available later this summer from this analysis.

Other EMSL short term work being done on CO monitoring include a contract with Sciences Application Inc. who has outfitted nine people in the Los Angeles area with CO monitors and recorded their CO exposure as they went about their normal activities. Data from this study should be available later this year.

EMSL is also planning a longer term CO monitoring study which will include an assessment of people's activities as well as their CO exposure. A diary type log will be kept by the people carrying the monitors to record their activities. Thus, a relationship can be developed between personal activity and CO exposure. It is tentatively expected that about 100 people will carry the monitors for certain time periods. This project will give OMSAPC a more definitive assessment of actual CO exposure as compared to that predicted from the fixed site NAAQS monitors. This work is starting in FY81 and will continue into FY82.

Some limited FY81 work is underway to improve CO personal monitors and to incorporate integrators in them so that both instantaneous CO and time integrated CO measurements can be taken.

B. Fuel Registration and Analysis

EMSL has the responsibility for operation of the fuel registration program required by Section 211(b) of the Clean Air Act Amendments. This program involves registration of all fuel and fuel additives listing their components, concentration levels, and purpose of using the additives. Much of the information (especially on fuel additive composition) can be held "confidential" by the provisions of these regulations. EMSL plans to issue a report on the fuels and fuel additives registered in late 1981.

Other provisions of Section 211 require EPA to promulgate regulations requiring needed health or emission-control-device testing of fuels and fuel additives. Initially, ORD was developing these regulations but in early 1980 the responsibility for proposing these regulations was shifted to OMSAPC. OMSAPC is still counting on ORD to develop a tier bioassay test for mutagenic compounds; this work is discussed later in Section IVC. OMSAPC will use these tier bioassay tests as one element in the fuel and fuel additive protocols.

EMSL also provided analysis support to the Office of Enforcement on trace contaminants in unleaded fuel such as lead, manganese and phosphorus. EMSL also analyzed some fuel samples for OMSAPC, performed benzo(a)pyrene analyses on Diesel particulate samples, and ran sample extractions prior to Ames test analysis. However, EMSL management decided the priority for this work was low and recently discontinued these analyses.

## IV Health Work

The ORD-HERL Laboratories in Cincinnati and Research Triangle Park provide OMSAPC with research support in the mobile source health area. The current work is on Diesels and development of a tier biossay test.

### A. Diesel Work

The largest amount of HERL FY81 mobile source resources are being spent on Diesel health work. The work includes short term bioassay tests, skin tumorigenesis, intratracheal instillation, and whole animal inhalation. This work is being used to determine a carcinogenic potency for Diesel exhaust particulate to use in a carcinogenic risk assessment. The end product of all of the Diesel health work will be a cancer risk assessment predicting the carcinogenic risk associated with exposure to light and heavy duty Diesel exhaust. The health work on Diesels is discussed below but the Diesel risk assessment is covered in Section V as a separate entity.

#### 1. Bioassay Tests

For several years HERL-RTP has been running a variety of short term bioassay tests including the Ames test, sister chromatid exchange, L-5178Y mouse lymphoma, and Balb tests. Two short term carcinogenesis tests (viral enhancement and Balb) have also been run.

These tests have been run on a variety of light and heavy duty Diesel particulate extracts. The light duty Diesels tested include an Oldsmobile, VW Rabbit, and Nissan. The Nissan Diesel had a poorly designed injector (which has been redesigned on newer Nissan Diesels) that resulted in considerable "after injection" (i.e. late injection) of Diesel fuel. EPA understands that Ames test results with the new injection system are considerably lower. The samples from the Caterpillar heavy duty Diesel were stored for an extended period which may have resulted in sample deterioration. OMSAPC has requested that

ORD run new heavy duty Diesel samples. Bioassay tests have also been run on particulate extract from a limited number of gasoline vehicles. The gasoline car tested was a 1977 Mustang catalyst equipped vehicle that apparently was running somewhat richer than manufacturers' specifications.

To date, very few if any bioassay tests have been run on gas phase samples from either gasoline or Diesel vehicles mainly due to lack of a method to collect samples for bioassay tests. However, as mentioned in Section II A, work is proceeding by ESRL to develop such a method and one is expected shortly.

The bioassay test response of the motor vehicle samples have been compared to other samples. The other samples collected are from coke ovens, roofing tar, and cigarette smoke condensate. The coke oven samples collected by ORD were taken from a coke oven owned by U.S. Steel in Gadsen, Alabama. The samples were collected in a location that was upwind of the coke ovens a large fraction of the time. However, it is thought that much of the sample mass was collected when the coke ovens were discharging and presumably the wind was in the right direction even though this may have occurred only a small fraction of the time. In other words, OMSAPC is not currently sure how much of the coke oven samples are actual coke oven emissions.[16] OMSAPC hopes that this point is clarified in the ORD report to be issued on this work. ORD is currently obtaining additional coke oven samples for bioassay and other tests. The additional samples are being obtained in the coke oven main itself and may give different results from the earlier samples. It is not known yet how the new samples would be representative of actual coke oven emissions to which people are exposed. The cigarette smoke condensate samples were obtained by a standard laboratory apparatus designed to duplicate cigarette smoking. The roofing tar emissions were obtained by collecting a particulate sample above a hot pot of roofing tar.

The reason that the coke oven, cigarette smoke, and roofing tar samples were investigated is that extensive epidemiology data exist for these substances while almost no good epidemiology data exist for Diesel emissions.

The results from the bioassay tests have been calculated as the response per unit mass of particulate extract. The responses were rated on a relative basis with the Nissan arbitrarily assigned a ranking of 100. A known carcinogen (benzo(a)pyrene) was used for comparison. The results from this work are summarized in Table 3 below and are discussed in detail in several references.[17,18,19,20] They were also covered in an ORD sponsored workshop held February 1981 on Diesel risk assessment.[16,21]

Table 3

COMPARATIVE POTENCY RANKINGS

| | MUTAGENESIS TESTS | | | | CARCINOGENESIS TESTS | | |
|---|---|---|---|---|---|---|---|
| | AMES[a] | SCE[a] | L-5178Y[a] | BALB[a] | VIRAL ENHANCE | BALB | TUMOR INITIATION[b] |
| DIESEL: CATERPILLAR | 4.3 | 0 | 1[c] | 0 | 0 | 0 | 0 |
| NISSAN | 100 | 100 | 100 | 100 | 100 | 100 | 100 |
| OLDS | 23 | 0 | 64 | 750 | 25 | 0 | 28 |
| VW RABBIT | 22 | 50 | 50 | NT[d] | 50 | NT | 6 |
| GASOLINE: | | | | | | | |
| MUSTANG | 25 | 1 | 38 | 750 | 50 | 200 | 16 |
| COMPARATIVE SOURCES: | | | | | | | |
| CIGARETTE | 7 | 0 | 21 | 300 | 200 | 200 | 0 |
| COKE | 18 | 44 | 339 | 15 | 800 | 500 | 355 |
| ROOF TAR | 7 | 291 | 850 | 750 | 2016 | 500 | 120 |
| STANDARDS: | | | | | | | |
| B(a)P | 1112 | 1750 | 189 | 25000 | 52000 | 16700 | 16500 |

[a]In the presence of an Aroclor-1254 induced rat hepatic S-9.
[b]Mouse skin tumor initiation in male and female sencar mice after 24 weeks of treatment.
[c]Testing incomplete at this time.
[d]Not tested.

-13-

The skin tumor initiation work is discussed later in this paper.

It was determined that the Balb test is not suitable for a complex mixture[16] so that the Balb data cannot be used for any comparisons. Also, there is some concern that the Ames test may overreact to nitrogen containing compounds such as are present in Diesel particulates. There is also concern about whether bioassay test results in general can be extrapolated to human health effects.[22]

Nevertheless, the data in Table 3 (especially the skin tumor initiation data) can be used as an input for a Diesel risk assessment.

HERL has also done some work to evaluate whether mutagens adsorbed on Diesel particulates are released in the presence of physiological fluids. This work showed that substantial mutagenic activity is released from Diesel particles upon incubation with serum and lung cytosol.[23] This work is continuing and is important in determining if physiological fluids present in the body alter the mutagenic activity of the particulate.

2. Skin Tumorigenesis and Intratracheal Instillation

HERL has started long term projects for skin tumorigenesis and intratracheal instillation.

The skin tumorigenesis work was done with motor vehicle and other samples as discussed in Section IVA1. Both C-57A and Sencar mice were used. Recent results with the C-57 black mice (a relatively non-sensitive breed) were negative (i.e. no carcinogenic response) with the exception of one dose of the roofing tar samples.[24] Work with the more sensitive Sencar mouse does show a positive response after 24 weeks of treatment.[25,26] These results are given in Table 3 above. These results are preliminary ones; the results from the full scale study should be available within a year. OMSAPC feels that additional work is needed for heavy duty Diesel samples. Also, the cigarette smoke condensate showed no result probably due to the fact that the active components are too dilute in the condensate sample. This work should be repeated with a more active fraction of the condensate.

It is generally thought[16] that the skin tumorigenesis results are more significant from a human health viewpoint than bioassay tests since the whole animal is involved.

The intratracheal instillation work with Syrian golden hamsters involves placing specific quantities of the samples listed in Table 3 on the trachea. The doses are repeated at certain intervals. The advantage of intratracheal instillation versus inhalation testing is that larger doses can be administered to the animals in a very controlled fashion. The animals are then followed over their lifetime (about 2 years) to see if cancerous tumors develop.

This work is being done under an EPA Cooperative Agreement with IITRI in Chicago. Unfortunately, there is probably no way to get any meaningful results until the animals die since this is a lifetime study. A report on the work is scheduled for early 1983.

3. Whole Animal Inhalation

HERL-Cincinnati has done extensive work on whole animal inhalation for Diesel exhaust. This work was described in detail in the recent proceedings of the EPA Diesel symposium held in 1979.

A study[28,29] was recently completed which examined the effect of Diesel particulate exposure on the ability of A/Jax mice to dispose of radioactive labelled BaP that were deposited by intratracheal instillation. While it was seen that mice exposed to Diesel exhaust had less capacity to dispose of the BaP, the significance of this finding is not clear to OMSAPC.

Another animal inhalation program involved the liver foci; test were run on rats that had most of their livers surgically removed and were fed a special choline-devoid or supplemented diet for 3-6 months. The rats were exposed to either clean air or Diesel exhaust. This test showed no changes in the rats exposed to Diesel exhaust.[30]

An initial study was done with Strain A mice which did seem to show a slight effect due to Diesel particulates but there were some questions about the significance of the results.[27,31] This study was repeated with more animals and the results are just being compiled now.

Another study[32] was performed with hamsters. A sample of Diesel particulate extract was intratracheally instilled in the animals. Lung tissues from these animals were later analyzed for chromatid exchange. Recent analysis[33] shows the response from this test is linearly related to Diesel particulate concentration. Thus, this test could be regarded as a useful in-vivo test. Future work could involve using other samples including more Diesels and possibly coke oven, cigarette smoke, and roofing tar samples.

Another project that is currently underway is intraperitoneal injection of Diesel particulate, coke oven, cigarette smoke condensate and roofing tar samples into the lung cavities of laboratory animals. This work will be a supplement to the other work on these samples discussed in Table 3.

Another project[34] involved determining if Strain A mice exposed to Diesel exhaust had abnormal sperm. No sperm abnormalities were noted in the Strain A mice. Also, the urine of Swiss mice exposed to Diesel exhaust was analyzed to see if it were positive on the Ames test due to excretion of mutagenic substances.[35] No difference was found in the urine of mice exposed to Diesel exhaust compared to the control group.

Finally, HERL has run a study[36] with male Chinese hamsters exposed to Diesel exhaust for six months. An increase in sperm abnormality was noted although no increase in chromosomal abnormalities in bone marrow cells were found in animals exposed to Diesel exhaust. Also, no increase in sister chromatid exchange was noted in bone marrow cells of these animals.

Other Diesel whole animal inhalation work is underway with cats, rabbits[37], rats[38] and hamsters. The project with cats is directed more towards non-carcinogenic lung effects.

OMSAPC has requested an evaluation from ORD on the general usefulness of inhalation studies to detect increased incidence of Diesel cancer in laboratory animals. EPA wants to be certain that a negative result on a whole animal inhalation test is in fact significant.

B. Carbon Monoxide Health Work

EPA has compiled a detailed summary of health effects work on carbon monoxide in the recent Criteria Document.[39] Of particular interest to EPA are the numerous studies[40,41,42,43,44,45,46,47] which show a relationship between carbon monoxide and the onset of heart pain in persons with angina pectoris. This work has been criticized since the onset of heart pain can be regarded as a subjective measure that may not be indicative of further heart damage. Also, OMSAPC has expressed interest in some studies[48,49] that have related CO levels in the ambient air to an increase in hospital admissions due to cardiorespiratory complaints. These studies have been criticized for several reasons (basically a lack of showing that the CO exposure itself was responsible for the health problems requiring hospital admission) and OMSAPC has expressed an interest in having them repeated. These and other health studies have been summarized in a recent review article.[50]

Both OMSAPC and the Office of Air Quality Planning and Standards (OAQPS) have expressed a need for additional CO health work. ORD is implementing FY81 programs to meet these needs.

The first program is a replication of the CO clinical studies relating CO exposure to actual changes in heart parameters during exercise for people with angina pectoris. This work will be done with a radionuclide gamma camera designed to determine changes in heart parameters (e.g. chamber discharge volume and rates). While this work is to be completed by December, 1982, OMSAPC has requested a written progress report in 1981.[51] Other CO work being done by HERL includes some clinical work to examine the Coburn equation which predicts the blood carboxyhemoglobin level as a function of CO exposure. This work will be completed in September 1982.

OMSAPC and OAQPS feel that work on the effect of CO on susceptible subgroups such as fetuses is needed. HERL has started some work on pregnant rats to determine if exposure to CO affects the newly born rat neonate. An interim report on this work which is now underway is to be prepared later this year. The study will be completed in 1982. HERL also plans some animal studies to determine the effect of CO alone and in combination with $O_3$ and $NO_2$ on xenobiotic metabolism in animals. Additional work is being considered to correlate CO exposure and fetal carboxyhemoglobin levels with cardiovascular and central nervous system maldevelopment in the animal fetus and neonate. These two projects would be complete in 1983.

HERL has proposed some epidemiology feasibility studies on CO but these studies have not been currently approved by the Mobile Source Research Committee. The one study would determine cardiovascular effects of CO exposure at high altitude while the second study would determine the effect of ambient carbon monoxide exposure on pregnancy outcome.

C. Tier Bioassay Development

OMSAPC has need for a tier bioassay test for both the Section 211 fuel and fuel additive regulations and the Section 202 (a)(4) regulations designed to assure that unregulated emissions from motor vehicles do not cause or contribute to an unreasonable risk to public health, welfare or safety. These two sets of regulations are being currently developed by OMSAPC. The tier bioassay test would be designed to minimize the cost and complexity of determining the potential carcinogenicity of these emissions. The test is designed in a tier fashion so that if a mixture passes the first test (a test that should result in few false negatives), no further testing is needed. If it fails, successive tests of increasing complexity are needed until the mixture passes a test (again a test designed for few false negatives). If a mixture fails the tier bioassay process, either the fuel/fuel additive for Section 211 or the vehicle for Section 202 (a)(4) may have to be altered until the emission products do pass the tier tests.

A problem with developing a tier test is to specify suitable pass/fail criteria for each successive test of the tier. HERL feels that they may not be in a position to specify these cut points. Yet, OMSAPC feels that HERL's specifying such cutpoints is an inherent part in developing this tier approach.

The following tests are being used by HERL:

1. microbial mutagenesis
2. cellular toxicity
3. gene mutations in mammalian cells
4. mitotic recombination in yeast
5. sister chromatid exchanges in mammalian cells
6. oncogenic transformation in mammalian cells
7. in vivo/in vitro confirmatory assays employed as required.

Endpoints include:

> gene mutations, DNA damage, chromosomal effects, oncogenic transformation (carcinogenesis, in vitro), and cellular toxicity.

A report on the short-term bioassay results and a report on correlation of short-term bioassay test results is due October 1981. A revised tier will be constructed and investigated with a final tier and guidelines being available for OMSAPC in late 1982. It is not known yet how toxic (i.e. non-cancer) emissions will be handled for Section 211 but it is possible that the approach that would be used for Section 202 (a)(4) could be used here as well. In addition to developing the tier biossay, HERL is running limited bioassay tests to support ESRL for the work described in Section II. HERL is also advising OMSAPC on the use of the Ames test for OMSAPC programs. While OMSAPC now has contractor capability to run the Ames test, HERL is still working on data analysis for the Ames test. This capability is in the process of being transferred to OMSAPC.

V. Diesel Carcinogenic Risk Assessment

The major output of the massive ORD Diesel research program is to be a revised carcinogenic risk assessment projecting the potential number of cancer cases that could be associated with exposure to light and heavy duty Diesel exhaust.

An initial risk assessment[52] was based on assuming the potency of Diesel exhaust extract is equivalent to that of the benzene-soluble organics from coke oven emissions. The best estimate number of projected cancer cases in 1990 would be about 350 and 670 for light and heavy duty Diesels respectively. Since this risk assessment was released, a number of criticisms of it have been made by various people including the National Academy of Sciences.

It is agreed that a new risk assessment is needed. ORD is scheduled to release a new risk assessment shortly. The biggest change that could be expected in the new risk assessment is that a lower potency would be assumed for Diesel exhaust compared to coke oven emissions based on the recent skin tumorigenesis results.[25,26,54]

ORD did release a report recently of great value to OMSAPC on the London Transit Worker study.[54] This epidemiology study of London transit workers exposed to Diesel bus emissions has been cited by many people as a strong indication that Diesel emissions result in no excess cancer risk. ORD analyzed the study parameters statisically looking at the particle level exposure time, and number of people involved. It was their conclusion that thousands of excess cancer deaths could result in the United States population as a whole and still be consistent with the results of the London Transit Worker study. The ORD analysis has been reviewed both inside and outside EPA by various experts.

## VI Health Effects Institute

In late 1980, the Health Effects Institute was formed to conduct health research for mobile source emissions. While the Institute is jointly funded by the industry and EPA, it is set up as an independent entity so that the research done will be purely scientific and free of any bias.

At this point, the Health Effects Institute is formulating plans for the type of research they would implement. EPA and the industry will be specifying the type of work that is important from their perspectives. Specific projects should be started later this year.

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